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Do salivary and serum collagenases have a role in an association between obstructive sleep apnea syndrome and periodontal disease? A preliminary case–control study

Nejat Nizam [a], Ozen K. Basoglu [b], Mehmet S. Tasbakan [b], Anna Holthöfer [c], Taina Tervahartiala [c], Timo Sorsa [c,d], Nurcan Buduneli [a,*]

[a] Department of Periodontology, School of Dentistry, Ege University, İzmir, Turkey
[b] Department of Chest Diseases, School of Medicine, Ege University, İzmir, Turkey
[c] University of Helsinki, Institute of Dentistry and Department of Oral and Maxillofacial Diseases, Helsinki University Hospital, Helsinki, Finland
[d] Division of Periodonlogy, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden.

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ABSTRACT

Objectives: Despite increasing evidence for an association of obstructive sleep apnea syndrome (OSAS) and periodontal disease, the pathophysiological linking mechanisms remain unclear. This study aims to evaluate the salivary and serum matrix metalloproteinase-2, -8, -9 (MMP-2, -8, -9), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), myeloperoxidase (MPO), neutrophil elastase (NE), neutrophil gelatinase-associated lipocalin (NGAL), as well as degree of activation of MMP-2, -9 of patients with and without OSAS.

Design: A total of 50 individuals were included in the study. There were 13, 17 and 20 individuals, respectively in the control (non-OSAS) group, mild-to-moderate OSAS and severe OSAS groups. Saliva, serum samples and clinical periodontal parameters were collected. Biofluid samples were analysed by immunofluorometric assay (IFMA), enzyme-linked immunosorbent assay (ELISA), western immunoblotting and gelatine zymography. Statistical analyses were performed using D'Agostino–Pearson omnibus normality test, Kruskal–Wallis test and Spearman rho rank correlation analysis.

Results: There were no statistically significant differences in clinical periodontal parameters between the study groups. Salivary NE and proMMP-2 levels were significantly lower in the OSAS groups than the control group ($p < 0.05$). Serum proMMP-9 concentration and the degree of MMP-9 activation in saliva were significantly lower in the severe OSAS group than the control group ($p < 0.05$). There were significant correlations between salivary and serum proMMP-9 and -2 concentrations ($p < 0.05$). Serum proMMP-2, NE and salivary proMMP-9 and -2 negatively correlated with indicators of OSAS severity ($p < 0.05$).

Conclusions: The present findings do not support a pathophysiological link between the severity of OSAS and clinical periodontal status via neutrophil enzymes or MMPs.

* *Corresponding author at*: Ege University, School of Dentistry, Department of Periodontology, 35100 Bornova, I˙zmir, Turkey. Tel.: +90 232 3881105; fax: +90 232 388 03 25.
E-mail address: nurcan.buduneli@ege.edu.tr (N. Buduneli).

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1. Introduction

Obstructive sleep apnea syndrome (OSAS) is a common and treatable disorder, which involves upper airway collapse during sleep and results in intermittent hypoxaemia and sleep fragmentation. An estimated prevalence of 4% in middle-aged males and 2% in middle-aged females has been reported.[1] There is increasing evidence to support that OSAS is an independent risk factor for cerebrovascular and cardiovascular disorders including congestive heart failure, hypertension, cardiac arrhythmias, myocardial infarction, cardiac arrhythmias, and stroke.[2–4]

The apnea-hypopnea index (AHI) is used to diagnose OSAS and it is calculated as the number of apneas and hypopneas per hour during sleep. AHI score of $\geq$5 with clinical symptoms such as witnessed apneas, excessive daytime sleepiness, loud snoring and nocturnal choking or AHI $\geq$ 15 without accompanying clinical symptoms is diagnosed as OSAS.[5] An AHI score <5 is accepted within normal limits, and scores between 5–15, 15–30, and $\geq$30 indicate mild, moderate, and severe OSAS, respectively.[6] OSAS is associated with systemic inflammatory response and risk factors of periodontitis including age, gender, obesity, smoking and diabetes[7–9] are shared by OSAS. Periodontitis is also associated with systemic inflammation and has been suggested to have a role in development of cardiovascular, cerebrovascular and pulmonary diseases, as well as complications of pregnancy and diabetes.[10,11] These associations have been suggested to proceed through inflammatory pathways[12] and with systemically increased levels of inflammatory markers due to periodontal disease.[13,14] Recently published data by Seo et al.[15] provided support for an association between OSAS and periodontitis.

During inflammation neutrophils are stimulated to release their enzymes, such as matrix metalloproteinases (MMPs), neutrophil elastase (NE) and myeloperoxidase (MPO).[16] MMPs are zinc-dependent endopeptidases, known for their ability to cleave several constituents of the extracellular matrix (ECM). Zymogen forms of the MMPs (proMMPs) are secreted from a large number of cell types into the matrix and activation of the proMMPs can result in discrete alterations in tissue architecture.[17] NE is a destructive proteolytic enzyme that is increased in patients with chronic inflammation; such as chronic obstructive pulmonary disease[18] and can cause connective tissue destruction by digesting several types of ECM proteins.[19] Myeloperoxidase (MPO), another destructive enzyme expressed by neutrophils, has a role in the oxygen dependent killing mechanism of the host immune system[20] and its increased salivary levels have been reported in patients with OSAS.[21] This peroxidase enzyme is also capable of modifying acute and chronic inflammatory reactions, and activating proMMP-8, -9, and inactivating TIMP-1.[22,23] Besides these enzymes, neutrophils also contain neutrophil gelatinase associated lipocalin (NGAL), a 25-kDa secretory glycoprotein, which is widely considered as an excellent indicator of acute and chronic kidney injury and positively associated with the severity of OSAS.[17]

It is hypothesized that increased levels of inflammatory biomarkers in patients with OSAS may also affect periodontal health. Therefore, this preliminary study is undertaken to investigate serum and salivary levels of MMP-2, -8 and -9, TIMP-1, MPO, NE, NGAL, degree of activation of MMP-2 and -9 in patients with and without OSAS and to investigate whether these biochemical parameters are related with severity of OSAS and/or clinical periodontal status.

2. Materials and methods

2.1. Study population

A total of 50 patients (20 females and 30 males; age range: 21–64 years) referred to Department of Chest Disease, School of Medicine, Ege University with complaints of sleep apnea-related symptoms were included in the present study between January 2011 and September 2012. The patients were monitored overnight in-laboratory polysomnography (Compumedics E Series, Australia or Alice 5 Diagnostic Sleep System, Philips, Respironics, USA) and AHI scores were used to determine the presence and severity of OSAS.[6,24] The patients were assigned into three groups as follows; 13 patients (8 females and 5 males; age range: 21–59 years) in the control group with AHI scores <5 and diagnosed as primary snoring (non-OSAS); 17 patients (8 females and 9 males; age range: 29–64 years) in the mild-to-moderate OSAS group with AHI scores between 5 and 30, and 20 patients (4 females and 16 males; age range: 26–61 years) in the severe OSAS group with AHI scores $\geq$30. The study was approved by the Ethics Committee of Ege University, School of Medicine and conducted in full accordance with ethical principles, including the World Medical Association's Declaration of Helsinki, as revised in 2000. The aims and methods of the study were thoroughly explained and written informed consent was received from each person before their enrolment in the study.

Demographic data including age, gender, smoking status, alcohol consumption and psychotropic drug use, anthropometric measurements such as height, weight, body mass index, circumferences of neck, waist and hip, and medical histories were evaluated. Chest X-ray, arterial blood gas analysis and pulmonary function tests were also performed. Smoking status was determined by self-reporting, but smokers or former-smokers were not excluded from the study. Patients with medical disorders, such as immunological disorders, diabetes mellitus, those who received antibiotic treatment within the last 3 months, and periodontal treatment within the last 6 months, or had less than 20 teeth and those wearing removable dentures were also excluded.

2.2. Saliva and serum sampling

All patients were asked to simply expectorate into polypropylene tubes in order to collect unstimulated whole saliva samples as described previously by Navazesh.[25] Saliva sampling was performed before clinical periodontal measurements and/or any periodontal intervention, in the morning following an overnight fast during which patients were asked not to drink anything except water or chew gum. 500 μL

aliquots of saliva samples were placed in sterile polypropylene tubes following clarification by centrifugation (800 × *g*) for 10 min at room temperature.

Five millilitres of venous blood were taken from the antecubital vein by a standard venipuncture method. The serum was separated by centrifugation at 1500 × *g* for 10 min and 500 μL amounts were aliquoted in sterile Eppendorf tubes. All biofluid samples were snap frozen and stored at −80 °C until the laboratory analysis and thawed immediately before starting the biochemical assays.

2.3. Clinical periodontal measurements

All clinical periodontal measurement including dichotomous plaque index (PI)[26] (as present or absent), probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP; as present or absent within 15 s after periodontal probing) were performed by a single calibrated examiner (NN) who was blinded to the sleep apnea variables and apnea status of the subjects. The measurements were performed using a periodontal probe (Williams periodontal probe, Hu-Friedy, Chicago, IL, USA) at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual locations) on each tooth, except the third molars. The intra-examiner reliability was assessed by an intraclass correlation coefficient of 0.90 for CAL and PD measurements.

2.4. MMP-8 analyses by immunofluorometric assay (IFMA)

MMP-8 levels in serum and saliva samples were analysed by a time-resolved IFMA using the methods previously described.[27,28] MMP-8 specific antibodies 8708 (Medix Biochemica Oy Ab, Kauniainen, Finland) were used as the catching and 8706 (Medix Biochemica Oy Ab, Kauniainen, Finland) as the tracer antibody, which was labelled using europium-chelate. Samples were diluted in assay buffer consisted of 20 mM Tris–HCl (pH 7.5), 0.5 M NaCl, 5 mM $CaCl_2$, 50 μM $ZnCl_2$, 0.5% bovine serum albumin, 0.05% sodium azide, and 20 mg/L diethylenetriaminepentaacetic acid and incubated for 1 h, followed by incubation with the tracer antibody for 1 h. Fluorescence was measured using a fluoremeter, 5 min after the addition of enhancement solution (1234 Delfia Research Fluoremeter, Wallac, Turku, Finland). The specificity of the monoclonal antibodies (27) against MMP-8 was the same as that of polyclonal MMP-8 antibodies.[27,29]

2.5. TIMP-1 analysis by enzyme-linked immunosorbent assay (ELISA)

TIMP-1 levels in saliva and serum samples were determined as described previously[30] using commercially available ELISA kits (Duoset ELISA Development Systems, R&D Systems, Minneapolis, USA). All samples were assayed in duplicate. The ELISA kit was capable of detecting the active, pro-, complexed and fragmented forms of the studied TIMP-1 as mentioned by the manufacturer. MMP-8/TIMP-1 ratios were also calculated as described previously.[28]

2.6. MPO and NE analysis

MPO (Immunodiagnostic AG, Bensheim, Germany) and NE (Bender MedSystems mbH, Vienna, Austria) levels in saliva and serum samples were measured according to the manufacturers' instructions. The secondary antibody in each kit was conjugated with horseradish peroxidase, and tetramethyl benzidine was used as the substrate.[30]

2.7. Analysis of MMP-2, -9, degree of their activation and NGAL zymography

Saliva and serum samples were run under non-reducing conditions on 7.5–10% gradient SDS-polyacrylamide gels impregnated with 1 mg/mL gelatin labelled fluorescently with 2-methoxy-2,4-diphenyl-3(2H)-furanone (Fluka, Sigma–Aldrich, Buchs SG, Switzerland). After washing, the gels were incubated in 50 mM Tris–HCl, pH 7.8, containing 150 mM NaCl, 1 mM $CaCl_2$, 1 μM $ZnCl_2$, and 0.02% NaN_3 for 48 h at 37 °C and stained with Coomassie Brilliant Blue. The total MMP content and the proportions of proforms and active forms of MMP-2, -9 and the degree of their activation as well as NGAL were estimated with the Bio-Rad Model GS-700 Imaging Densitometer using the Quantity-program (Bio-Rad Laboratories, Hercules, CA, USA).[31,32]

2.8. Western immunoblotting

Salivary and serum samples were separated by SDS-polyacrylamide gel electrophoresis on 7.5–10% gels under non-reducing conditions and transferred electrophoretically to nitrocellulose filters. The filters were incubated with polyclonal or monoclonal antibodies against MMP-2, -9 or NGAL.[33] The antigen–antibody complexes were visualized with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminesence (ECL) detection system according to manufacturer's instructions (Amersham Pharmacia Biotech Inc., NJ, USA).[31]

2.9. Statistical analyses

A statistical software program (GraphPad Prism version 6.00c for Mac OS X, GraphPad Software, California, USA) was used for the statistical analyses. The distribution of the variables was validated by D'Agostino–Pearson omnibus normality test, the differences between groups were evaluated using Kruskal–Wallis test, and Dunn's test was used to correct for multiple comparisons. Correlations between clinical and biochemical data were assessed by Spearman rho rank correlation analysis. All the tests were performed at $\alpha = 0.05$ significance level.

3. Results

Demographic data and clinical periodontal parameters of the patients are presented in Table 1. The clinical periodontal parameters revealed higher values in the severe OSAS group, but none of the differences reached the level of statistical significance ($p > 0.05$). The female to male ratio

Table 1 – Demographic variables and periodontal parameters of the patients. Values are given as mean ± standard deviation.

Clinical variable	Control	Mild-to-moderate OSAS	Severe OSAS
Age (years)	43.23 ± 9.08	49.88 ± 11.47	46.00 ± 9.94
Gender (F/M) (*n*)	8/5	8/9	4/16***
Smoking (non-smoker/smoker/ex-smoker)	4/5/4	12/3/2	11/5/4
No. of teeth present	24.54 ± 3.23	25.29 ± 3.31	26.10 ± 2.36
PD (mm)	2.05 ± 0.45	2.41 ± 0.49	2.56 ± 0.81
CAL (mm)	2.22 ± 0.63	2.68 ± 0.72	3.08 ± 1.32
PI	1.44 ± 0.55	1.27 ± 0.55	1.71 ± 0.83
Sites with PD > 4 mm (%)	2.10 ± 5.05	7.02 ± 11.21	12.07 ± 14.26
BOP (%)	24.73 ± 17.63	33.11 ± 16.65	35.97 ± 24.12
AHI (events/h)	2.64 ± 1.82*	17.24 ± 7.90	67.49 ± 30.39**
BMI (kg/m^2)	31.71 ± 4.56	31.85 ± 5.32	34.18 ± 7.24

* Significantly lower than the mild-to moderate ($p = 0.016$) and severe ($p < 0.0001$) OSAS groups.
** Significantly higher than control ($p < 0.0001$) and mild-to moderate ($p = 0.004$) OSAS groups.
*** Significantly lower than the control group ($p = 0.024$).

was significantly lower in the severe OSAS group than the control group ($p = 0.024$).

Serum proMMP-9 levels in the severe OSAS group were significantly lower than the control group ($p = 0.046$), whereas, no significant differences were found between the control and the mild-to-moderate OSAS groups or between the two OSAS groups (Fig. 1). No statistically significant difference was detected in serum MMP-8, TIMP-1, NE, MPO, proMMP-2 concentrations or MMP-8/TIMP-1 ratio, between the study groups ($p > 0.05$). NGAL, actMMP-2, actMMP-9, degree of MMP-2 and -9 activations were below the detection limits of the assay kits in all serum samples.

All of the assayed biochemical parameters in the saliva samples were above the detection limits of the assay kits. Salivary NE concentrations were significantly lower in both mild-to-moderate and severe OSAS groups than the control group ($p = 0.007$ and $p = 0.004$, respectively) (Fig. 2). There were no significant differences between the study groups in salivary concentrations of MMP-8, TIMP-1, MPO, NGAL or MMP-8/TIMP-1 ratio ($p > 0.05$). Salivary concentrations of proMMP-2 were significantly lower in the mild-to-moderate and severe OSAS groups compared to the control group (both $p = 0.002$) (Fig. 3). The degree of MMP-9 activation in saliva was significantly lower in the severe OSAS group than the control group ($p = 0.037$) and there was no significant difference between the two OSAS groups. Salivary concentrations of proMMP-9, actMMP-9, actMMP-2 and degree of MMP-2 activation were similar in the study groups ($p > 0.05$).

Correlations between salivary and serum biochemical data are presented in Table 2. Salivary and serum proMMP-9 levels correlated negatively ($r = -0.321$; $p = 0.032$), whereas proMMP-2 levels correlated positively ($r = 0.312$; $p = 0.037$).

The correlations between clinical periodontal parameters, indicators of OSAS severity and serum, salivary biochemical data are presented in Table 3. Oxygen desaturation index (ODI) negatively correlated with serum proMMP-2 levels ($r = -0.293$; $p = 0.045$) and AHI both with serum NE and proMMP-2 concentrations ($r = -0.323$; $p = 0.025$ and $r = -0.364$; $p = 0.011$, respectively). There was a significant negative correlation between the percentage of sleep time with pulse oximeter oxygen saturation (SpO_2) <90% and salivary proMMP-9 ($r = -0.306$; $p = 0.034$). AHI negatively correlated with salivary proMMP-9 and -2 ($r = -0.364$; $p = 0.010$ and $r = -0.326$; $p = 0.022$, respectively).

4. Discussion

In the present case-control study, salivary and serum levels of MMP-8, TIMP-1, MPO, NE, NGAL, degree of activation of MMP-2 and -9 were evaluated comparatively in mild-to-moderate OSAS, severe OSAS and non-OSAS control groups. Salivary[13,14] and serum biomarkers[14] may differ in patients with periodontal disease and especially salivary biomarkers may pose a useful tool to determine the presence or risk of periodontal disease. To the best of our knowledge, this is the first study evaluating salivary and serum proteolytic enzyme concentrations in regards with OSAS severity and clinical periodontal status.

Very few studies have searched a possible link between OSAS and periodontal disease. In a recent study, Seo et al.[15] investigated the possible association between OSAS and onset as well as progression of periodontal disease in a Korean population of 687 individuals. They performed standard polysomnography, clinical periodontal examinations and health-screening examinations. The authors proposed a causal relationship between these two conditions basing on the higher prevalence of periodontitis in patients with OSAS.[15] Keller et al.[34] reported a higher prevalence of prior chronic periodontitis in cases (individuals with OSAS) compared to controls (individuals without OSAS) in a Taiwan population and the authors suggested a higher risk of OSAS in patients with periodontitis. In the present study, the clinical periodontal parameters, particularly the number of sites with PD > 4 mm showed a tendency to increase from the control to the severe OSAS group with no statistically significant differences between the study groups.

Increased levels of NE were reported in gingival crevicular fluid samples of patients with periodontal disease[35] and NE appears to have a role in degradation of non-collagenous protein-covering collagen fibrils in the early destructive phases of periodontal disease.[36] Moreover, salivary NE activity was suggested to be associated with the severity of periodontitis.[37] Previous studies have demonstrated that OSAS is able

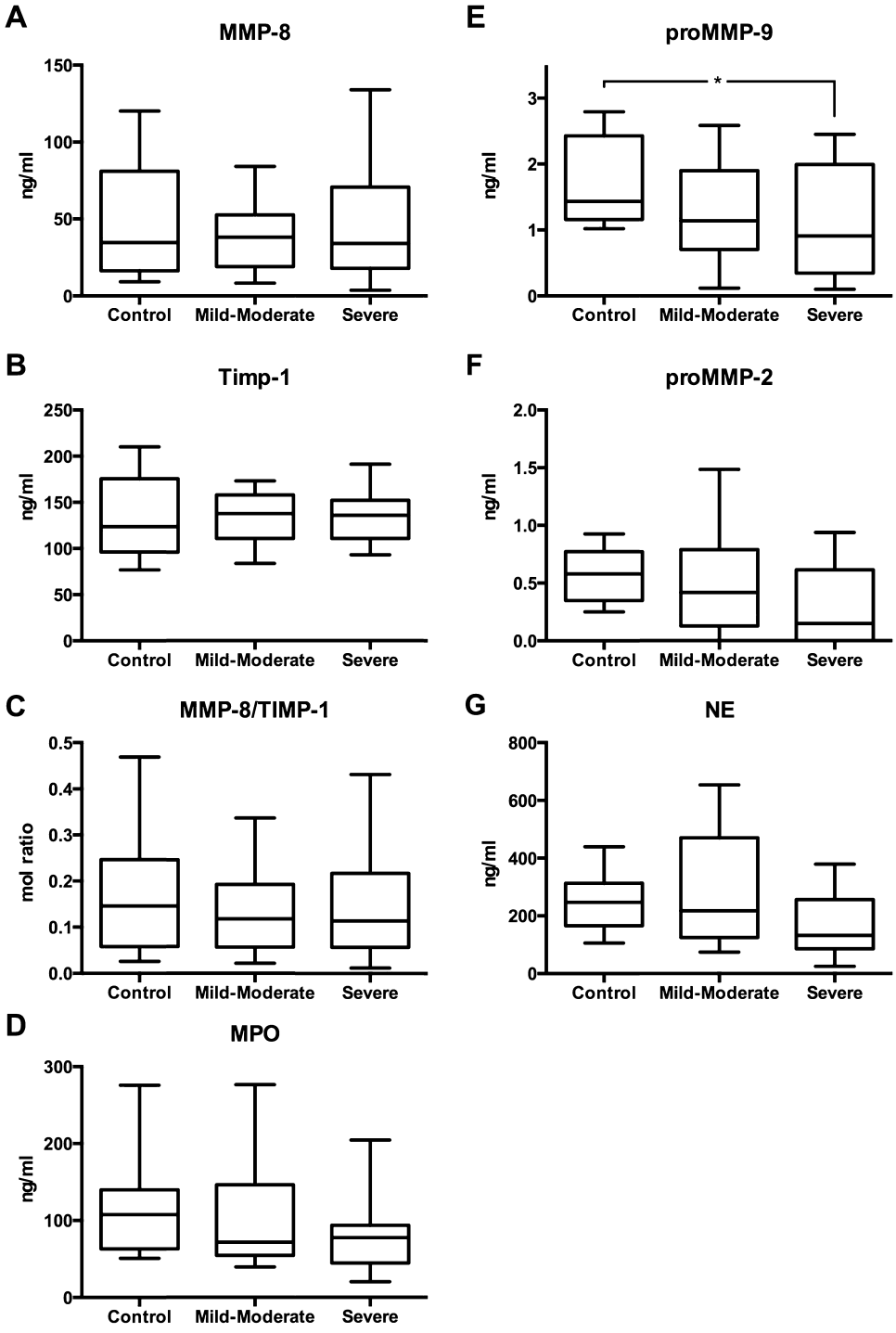

Fig. 1 – Serum levels of MMP-8, proMMP-9, proMMP-2, NE, MPO and TIMP-1 and the ratio of MMP-8/TIMP-1 in the study groups. Box and whiskers represent median, Q1, Q3 and min–max values and horizontal bars indicate the significant differences between the groups. Serum proMMP-9 concentrations were significantly lower in the severe OSAS group than the control group. There were no significant differences between the study groups in serum concentrations of MMP-8, NE, MPO, proMMP-2, TIMP-1 or MMP-8/TIMP-1 ratio ($p > 0.05$).

to activate circulating neutrophils[38] and increase plasma markers of systemic inflammation.[39,40] Higher levels of salivary and/or serum NE could be expected in patients with OSAS. However, the present study revealed rather unexpected findings as significantly lower salivary NE concentrations were found in the OSAS groups (both mild-to-moderate and severe groups) than the non-OSAS individuals and a negative correlation was detected between serum NE and AHI scores of the patients. The present serum data also indicated tendency of NE concentrations to decrease in patients with

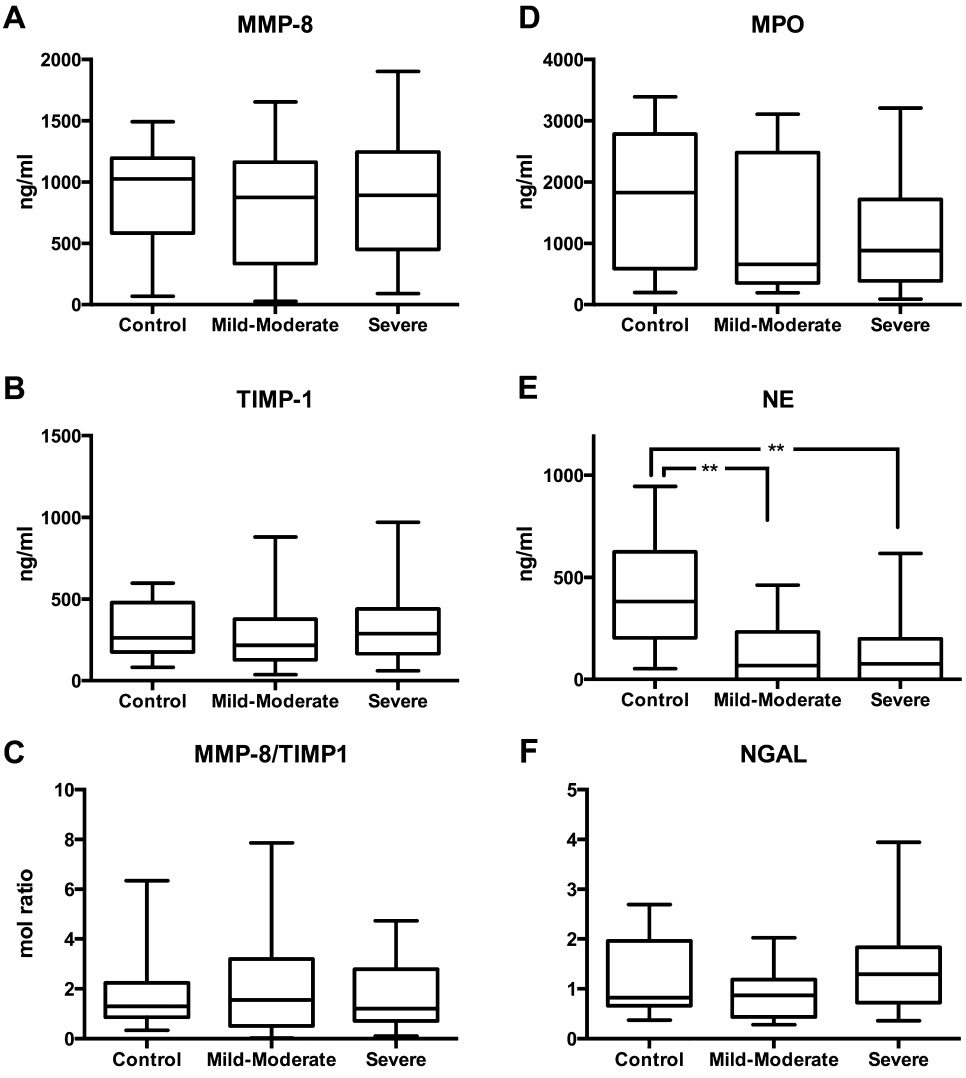

Fig. 2 – Salivary levels of MMP-8, MPO, NE, NGAL and TIMP-1 and the ratio of MMP-8/TIMP-1 in the study groups. Box and whiskers represent median, Q1, Q3 and min–max values and horizontal bars indicate the significant differences between groups. Salivary NE concentrations were significantly lower in both mild-to-moderate and severe OSAS groups than the control group ($p = 0.007$ and $p = 0.004$, respectively). Salivary concentrations of MMP-8, TIMP-1, MPO, NGAL and MMP-8/TIMP-1 ratio were similar in the study groups.

OSAS. Taken together with previous data, the present findings may suggest that OSAS may be responsible for not only increasing serum levels of inflammatory biomarkers, but also for decreasing salivary biomarkers such as NE. It is, yet to be clarified in larger scale studies whether the present finding is incidental.

OSAS is known to have several effects on neutrophils such as increasing their activation,[38] production of reactive oxygen species,[41] expression of adhesion molecules and decreasing apoptosis rates.[42] According to the present findings, there seems to be a tendency for decreased serum concentrations of NE, proMMP-2 and MPO from health to severe OSAS. The present negative correlation between serum NE and AHI scores of the patients also provide further support for an inverse relation between OSAS and serum NE concentration. It is likely that this finding may be related with local outcomes of OSAS rather than systemic consequences, as serum NE levels did not reveal significant differences between the present study groups. The failure of detection of many assayed biomarkers in the present serum samples may be regarded as a parallel finding.

Several members of the MMP family are involved in periodontal tissue destruction, including MMP-2, -8, and -9.[43,44] The present findings indicated significant differences in the degree of MMP-9 activation in saliva samples and serum proMMP-9 levels, both of which were significantly lower in the severe OSAS group than the control group. These findings may be interpreted as a negative effect of severe OSAS on serum proMMP-9 expression and its salivary activity. Ye et al.[45] and Chuang et al.[46] demonstrated increased serum MMP-9 levels in patients with OSAS, but none of these studies involve clinical periodontal examination. One possible explanation for the discrepancy between the findings of the previous studies and those of the present study may be the differences in

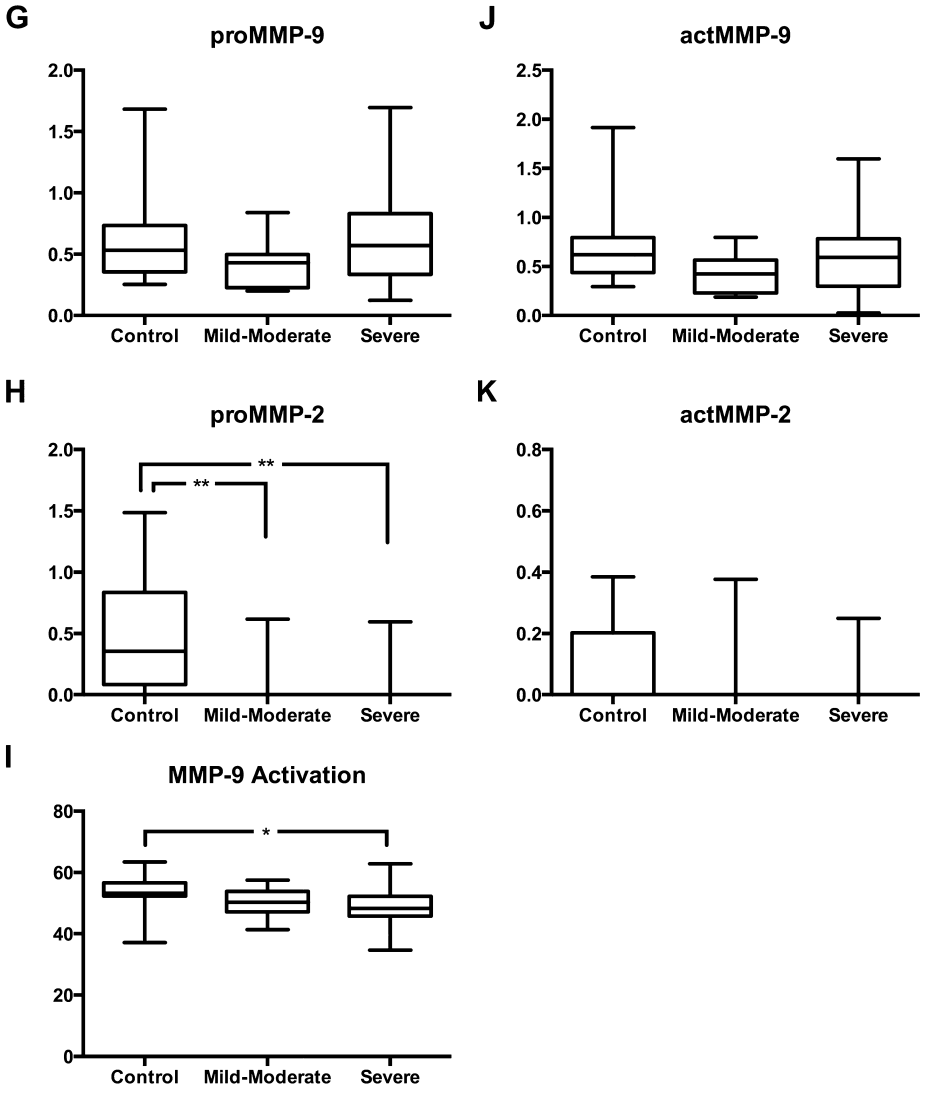

Fig. 3 – Salivary levels of proMMP-9, actMMP-9, proMMP-2, actMMP-2 and degree of MMP-9 activation in the study groups. Box and whiskers represent median, Q1, Q3 and min–max values and horizontal bars indicate the significant differences between the groups. Salivary concentrations of proMMP-2 were significantly lower in the mild-to-moderate and severe OSAS groups compared to the control group (both $p = 0.002$). The degree of MMP-9 activation in saliva was significantly lower in the severe OSAS group than the control group ($p = 0.037$). Salivary concentrations of proMMP-9, actMMP-9, actMMP-2 and degree of MMP-2 activation were similar in the study groups ($p > 0.05$).

demographic parameters as well as clinical periodontal status of the patients, as clinical periodontal parameters were not reported in previous studies.[45,46]

Salivary and serum concentrations of proMMP-2 and -9 levels correlated with each other suggesting that these biofluids are exposed to similar influences and salivary source of these zymogen MMPs could be related to serum or vice versa. The present data indicated negative correlations between ODI, AHI and serum proMMP-2, between AHI and saliva proMMP-2, -9 levels, and between $SpO_2 < 90\%$ and saliva proMMP-9 levels. These negative correlations may be explained by an inverse interaction between the levels of zymogen forms of MMP-2, MMP-9 in saliva, serum and the severity of OSAS.

Akpinar et al.[21] demonstrated increased salivary MPO concentration in patients with OSAS and positive correlation between AHI and ODI. Furthermore, Murase et al.[17] found positive correlation between serum NGAL and the severity of OSAS as determined by AHI, ODI scores. The present salivary MPO and NGAL data do not provide support for those of previous studies,[17,21] as salivary MPO levels were similar in patients with or without OSAS. Moreover, the present data revealed no significant correlations between NGAL, MPO levels and indicators of OSAS severity. Again, the differences in demographic variables as well as clinical periodontal status may explain the discrepancy between the present study and the previous ones, which do not involve any clinical periodontal examination.

One limitation of the present study could be the inclusion of current and former smokers, as smoking is known to affect salivary cytokine levels.[14] However, the current smoker/former smoker ratios were similar in the present study groups

Table 2 – Major correlations between serum and salivary biochemical data. Data without any significant correlation was excluded. Statistically significant correlations are presented in bold face.

Serum	Saliva				
	TIMP-1	ProMMP-9	ProMMP-2	MMP-9 activation	MMP-2 activation
MMP-8					
r	−0.329	0.031	0.012	−0.019	0.318
p	**0.027**	0.838	0.940	0.898	**0.028**
MPO					
r	−0.382	0.322	0.246	0.341	0.437
p	**0.010**	**0.031**	0.103	**0.018**	**0.002**
NE					
r	−0.168	0.188	0.189	0.430	0.412
p	0.271	0.216	0.214	**0.002**	**0.004**
Pro MMP-9					
r	0.006	−0.321	−0.355	−0.046	0.041
p	0.967	**0.032**	**0.017**	0.755	0.783
Pro MMP-2					
r	−0.063	0.284	0.312	0.480	0.715
p	0.681	0.058	**0.037**	**0.001**	**<0.001**

and a similar effect of smoking would be expected on the obtained biochemical data in each study group. Another likely limitation could be rather low number of patients (50 patients), which prevented comparisons between healthy, gingivitis and chronic periodontitis subgroups. One explanation for the rather low number of patients is the high frequency of various systemic diseases in this age group, as many of the screened patients did not fulfil the systemic and/or oral inclusion criteria.

Within the limits of the present study, it can be concluded that NE release in saliva is likely to decrease in OSAS independently from its severity. Furthermore, MMP-9 activation in saliva and proMMP-9 in serum seems to be negatively influenced by the severity of OSAS. The present findings, however, failed to demonstrate a pathophysiological link between the severity of OSAS and clinical periodontal status via neutrophil products and MMPs. The lack of significant differences in most of the assayed biochemical parameters in saliva/serum may also be related to the lack of statistically significant differences in clinical periodontal parameters between the study groups, although there seems to be a trend for deterioration in clinical periodontal status. Future studies with larger patient population, which investigate host cell-derived biomarkers together with microbiological factors, may better clarify the issue.

Table 3 – Major correlations of clinical periodontal parameters/indicators of OSAS severity with serum and salivary biochemical data. Data without any significant correlation was excluded. Statistically significant correlations are presented in bold face.

Clinical variable	Serum biomarkers						Salivary biomarkers			
	MMP-8/TIMP-1	MPO	NE	ProMMP-9	ActMMP-9	ProMMP-2	MMP-8	MPO	ProMMP-9	ProMMP-2
PD										
r	0.003	−0.110	−0.139	0.113	0.011	−0.300	0.267	0.218	−0.333	−0.335
p	0.983	0.456	0.346	0.444	0.941	**0.038**	0.063	0.132	**0.019**	**0.019**
CAL										
r	−0.025	−0.063	−0.123	0.187	0.093	−0.304	0.270	0.244	−0.349	−0.355
p	0.867	0.669	0.407	0.204	0.530	**0.036**	0.060	0.091	**0.014**	**0.012**
PI										
r	0.178	0.298	0.273	0.290	0.294	0.171	0.329	0.327	−0.088	−0.109
p	0.225	**0.040**	0.061	**0.046**	**0.043**	0.246	**0.021**	**0.022**	0.549	0.456
BOP										
r	0.312	0.275	0.090	0.119	0.124	−0.045	0.252	0.253	−0.076	−0.173
p	**0.031**	0.059	0.544	0.422	0.401	0.761	0.081	0.080	0.604	0.235
ODI										
r	0.016	−0.190	−0.274	0.059	−0.006	−0.293	0.130	−0.038	−0.267	−0.220
p	0.914	0.201	0.062	0.694	0.966	**0.045**	0.379	0.795	0.067	0.134
AHI										
r	−0.001	−0.200	−0.323	0.114	0.024	−0.364	0.025	−0.126	−0.364	−0.326
p	0.996	0.173	**0.025**	0.441	0.872	**0.011**	0.864	0.388	**0.010**	**0.022**
$SpO_2 < 90\%$										
r	0.058	−0.126	−0.174	0.138	0.031	−0.265	0.085	−0.084	−0.306	−0.270
p	0.700	0.399	0.242	0.356	0.836	0.072	0.565	0.569	**0.034**	0.063

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Conflict of interest

The authors report no conflict of interest related in the present study.

Ethical approval

The study has been approved by the Ethics Committee of Ege University, School of Medicine, with the reference number of 12/124.

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REFERENCES

1. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993;**328**:1230–5.
2. Bradley TD, Floras JS. Obstructive sleep apnoea and its cardiovascular consequences. *Lancet* 2009;**373**:82–93.
3. Selim B, Won C, Yaggi HK. Cardiovascular consequences of sleep apnea. *Clin Chest Med* 2010;**31**:203–20.
4. Bassetti CL, Milanova M, Gugger M. Sleep-disordered breathing and acute ischemic stroke: diagnosis, risk factors, treatment, evolution, and long-term clinical outcome. *Stroke* 2006;**37**:967–72.
5. American Academy of Sleep Medicine. International classification of sleep disorders. *Diagnostic and coding manual*. 2nd ed. Westchester, IL: American Academy of Sleep Medicine; 2005: 52–8.
6. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The report of an American Academy of Sleep Medicine Task Force. *Sleep* 1999;**22**:667–89.
7. Filoche SK, Cornford E, Gaudie W, Wong M, Heasman P, Thomson WM. Smoking, chronic periodontitis and smoking cessation support: reviewing the role of dental professionals. *N Z Dent J* 2010;**106**:74–7.
8. Arbes Jr SJ, Agustsdottir H, Slade GD. Environmental tobacco smoke and periodontal disease in the United States. *Am J Public Health* 2001;**91**:253–7.
9. Timmerman MF, van der Weijden GA. Risk factors for periodontitis. *Int J Dent Hyg* 2006;**4**:2–7.
10. Beck JD, Offenbacher S. Systemic effects of periodontitis: epidemiology of periodontal disease and cardiovascular disease. *J Periodontol* 2005;**76**(Suppl. 11):2089–100.
11. Dietrich T, Garcia RI. Associations between periodontal disease and systemic disease: evaluating the strength of the evidence. *J Periodontol* 2005;**76**(Suppl. 11):2175–84.
12. Moutsopoulos NM, Madianos PN. Low-grade inflammation in chronic infectious diseases: paradigm of periodontal infections. *Ann N Y Acad Sci* 2006;**1088**:251–64.
13. Gümüş P, Nizam N, Nalbantsoy A, Ozçaka O, Buduneli N. Saliva, serum levels of pentraxin-3 and interleukin-1beta in generalised aggressive or chronic periodontitis. *J Periodontol* 2014;**85**:e40–6.
14. Gümüş P, Nizam N, Lappin DF, Buduneli N. Saliva and serum levels of B-cell activating factors and tumor necrosis factor-alpha in periodontitis patients. *J Periodontol* 2014;**85**:270–80.
15. Seo WH, Cho ER, Thomas RJ, An SY, Ryu JJ, Kim H, Shin C. The association between periodontitis and obstructive sleep apnea: a preliminary study. *J Periodontal Res* 2013;**48**:500–6.
16. Nussbaum G, Shapira L. How has neutrophil research improved our understanding of periodontal pathogenesis? *J Clin Periodontol* 2011;**38**(Suppl. 11):49–59.
17. Murase K, Mori K, Yoshimura C, Aihara K, Chihara Y, Azuma M, et al. Association between plasma neutrophil gelatinase associated lipocalin level and obstructive sleep apnea or nocturnal intermittent hypoxia. *PLOS ONE* 2013;**8**:e54184.
18. El-Eshmawy MM, El-Adawy EH, Mousa AA, Zeidan AE, El-Baiomy AA, Abdel-Samie ER, et al. Elevated serum neutrophil elastase is related to prehypertension and airflow limitation in obese women. *BMC Womens Health* 2011;**11**:1–7.
19. Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, et al. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Ann Med* 2006;**38**:306–21.
20. Winterbourn CC, Kettle AJ. Redox reactions and microbial killing in the neutrophil phagosome. *Antioxid Redox Signal* 2013;**18**:642–60.
21. Akpinar ME, Yigit O, Altundag A, Demirel GY, Kocak I. Salivary and serum myeloperoxidase in obstructive sleep apnea. *J Otolaryngol Head Neck Surg* 2012;**41**:215–21.
22. Liu YC, Chung CJ, Shiue HS, Cheng YY, Huang SR, Su CT, et al. Genetic polymorphisms of myeloperoxidase and their effect on hypertension. *Blood Press* 2013;**22**:282–9.
23. Alfakry H, Sinisalo J, Paju S, Nieminen MS, Valtonen V, Tervahartiala T, et al. The association of serum neutrophil markers and acute coronary syndrome. *Scand J Immunol* 2012;**76**:181–7.
24. Iber C, Ancoli-Israel S, Chesson AL, Quan SF. *The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications*. Westchester, IL: American Academy of Sleep Medicine; 2007.
25. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci* 1993;**694**:72–7.
26. Quigley G, Hein J. Comparative cleansing efficiency of manual and power brushing. *J Am Dent Assoc* 1968;**65**:26–9.
27. Hanemaaijer R, Sorsa T, Konttinen YT, Ding Y, Sutinen M, Visser H, et al. Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cells. Regulation by tumor necrosis factor-alpha and doxycycline. *J Biol Chem* 1997;**272**:31504–9.
28. Tuomainen AM, Nyyssonen K, Laukkanen JA, Tervahartiala T, Tuomainen TP, Salonen JT, et al. Serum matrix metalloproteinase-8 concentrations are associated with cardiovascular outcome in men. *Arterioscler Thromb Vasc Biol* 2007;**27**:2722–8.
29. Lauhio A, Salo T, Ding Y, Konttinen YT, Nordström D, Tschesche H, et al. In vivo inhibition of human neutrophil collagenase (MMP-8) activity during long-term combination therapy of doxycycline and non-steroidal anti-inflammatory drugs (NSAID) in acute reactive arthritis. *Clin Exp Immunol* 1994;**98**:21–8.

30. Rautelin HI, Oksanen AM, Veijola LI, Sipponen PI, Tervahartiala TI, Sorsa TA, et al. Enhanced systemic matrix metalloproteinase response in *Helicobacter pylori* gastritis. *Ann Med* 2009;**41**:208–15.
31. Sorsa T, Salo T, Koivunen E, Tyynelä J, Konttinen YT, Bergmann U, et al. Activation of type IV procollagenases by human tumor-associated trypsin-2. *J Biol Chem* 1997;**272**:21067–74.
32. Paju A, Sorsa T, Tervahartiala T, Koivunen E, Haglund C, Leminen A, et al. The levels of trypsinogen isoenzymes in ovarian tumour cyst fluids are associated with promatrix metalloproteinase-9 but not promatrix metalloproteinase-2 activation. *Br J Cancer* 2001;**84**:1363–71.
33. Westerlund U, Ingman T, Lukinmaa PL, Salo T, Kjeldsen L, Borregaard N, et al. Human neutrophil gelatinase and associated lipocalin in adult and localized juvenile periodontitis. *J Dent Res* 1996;**75**:1553–63.
34. Keller JJ, Wu CS, Chen YH, Lin HC. Association between obstructive sleep apnoea and chronic periodontitis: a population-based study. *J Clin Periodontol* 2013;**40**:111–7.
35. Smith QT, Harriman L, Au GS, Stoltenberg JL, Osborn JB, Aeppli DM, et al. Neutrophil elastase in crevicular fluid: comparison of a middle-aged general population with healthy and periodontitis groups. *J Clin Periodontol* 1995;**22**:935–41.
36. Ujiie Y, Oida S, Gomi K, Arai T, Fukae M. Neutrophil elastase is involved in the initial destruction of human periodontal ligament. *J Periodontal Res* 2007;**42**:325–30.
37. Mantyla P, Buduneli E, Emingil G, Tervahartiala T, Pussinen PJ, Bariş N, et al. Acute myocardial infarction elevates serine protease activity in saliva of patients with periodontitis. *J Periodontal Res* 2012;**47**:345–53.
38. Dyugovskaya L, Lavie P, Lavie L. Increased adhesion molecules expression and production of reactive oxygen species in leukocytes of sleep apnea patients. *Am J Respir Crit Care Med* 2002;**165**:934–9.
39. Ohga E, Tomita T, Wada H, Yamamoto H, Nagase T, Ouchi Y. Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1. *J Appl Physiol* 2003;**94**:179–84.
40. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* 1997;**82**:1313–6.
41. Pilkauskaite G, Miliauskas S, Sakalauskas R. Reactive oxygen species production in peripheral blood neutrophils of obstructive sleep apnea patients. *Sci World J* 2013;**12**:4217–63.
42. Dyugovskaya L, Polyakov A, Lavie P, Lavie L. Delayed neutrophil apoptosis in patients with sleep apnea. *Am J Respir Crit Care Med* 2008;**177**:544–54.
43. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 1993;**4**:197–250.
44. Makela M, Salo T, Uitto V-J, Larjava H. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. *J Dent Res* 1994;**73**:1397–406.
45. Ye J, Liu H, Li Y, Liu X, Zhu JM. Increased serum levels of C-reactive protein and matrix metalloproteinase-9 in obstructive sleep apnea syndrome. *Chin Med J* 2007;**120**:1482–6.
46. Chuang LP, Chen NH, Lin SW, Chang YL, Chao IJ, Pang JH. Increased matrix metalloproteinases-9 after sleep in plasma and in monocytes of obstructive sleep apnea patients. *Life Sci* 2013;**93**:220–5.